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BULLETIN OF
THE NEW YORK ACADEMY
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NOVEMBER 1939

DISTRIBUTION OF ENZYMES IN TISSUE AND CELLS

Harvey Lecture, March 16, 1939

K. LINDERSTRØM-LANG*

THE relationship between the sciences, histology and biochemistry is a rather distant one, however intimately may be connected the phenomena they describe in nature itself. On one hand we have the histologist's microscopic picture of a piece of tissue or of a cell with all its mysteries: formed bodies, nuclei, granules of different kinds, etc. On the other hand we have the multitude of things which the cells can do; the metabolic processes going on in their interior. Between these two aspects of the cells and the tissue formed by them there is a gap which it is the task of the histochemist and the histophysiologist to fill. The histochemist is assumed to be interested mainly in the distribution of matter in relation to the histologist's structural picture of the cells, while the work of the histophysiologist is concerned mainly with how chemical processes may take place at a given distribution of chemical compounds. The boundary between the two fields is not a sharp one but it may be said that the greatest progress in histochemistry has occurred in studies on distribution of chemical compounds, the histochemistry above

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all being a topochemistry relating histological structure to chemical composition.

We may speak of a qualitative and a quantitative histochemistry. In qualitative histochemistry, developed in the histologists' laboratories, the absorption or refraction of light, caused directly or indirectly by the different constituents of a cell, is observed in a microscope, either without previous treatment of the cell or after a more or less vigorous fixation and combination of the constituents with other chemical compounds, especially dyes (For references see Lison[1]).

The quantitative histochemical methods are adapted mainly to the study of catalytically active constituents of the cell, i.e., its enzymes, and have features in common with ordinary biochemical technique. The elements of this procedure are the removal of small pieces of tissue or cells with known size and well defined histological structure and the investigation of these pieces by means of well-known chemical methods modified for the specific purpose of estimating small amounts of chemical compounds in small amounts of material.

This study of the quantitative topochemistry of enzymes is the subject of my lecture today. The problem is a fascinating, though very difficult one. The difficulties encountered are not so much connected with the chemical analysis of minute quantities of enzymes as with the histological side of the question. In other words, it is comparatively easy to find micromethods suitable for a rather detailed study of the distribution of enzymes, but it is extremely difficult to define, in a quantitative way, structural elements in cells or tissue to which the quantities of enzymes found may be referred. The ultimate aim of the histochemist interested in enzyme chemistry is to prove that certain portions of the cell – granules, vacuoles, nucleus, the cytoplasm proper – are or are not carriers of enzymatic activity. In general we must admit that we are far from a realization of this dream, in spite of the fact that more opinions have been expressed concerning this question than years of experimental work would justify; but even so, in a number of cases, the structure of tissue and cells may be sufficiently well defined to make profitable a comparison with the results of quantitative chemical analysis.

Firstly, cases may be mentioned where the question put to nature is simple enough to permit the histochemist to omit a closer cytological analysis. A problem belonging to this group is, for example, whether the two cells formed from a sea-urchin egg after one cleavage have the same

or different contents of a given enzyme per cell or per unit of volume measured under the microscope, or per unit of total nitrogen, determined by a micro-Kjeldahl method. Another example is the well-known problem of respiration in the animal and the vegetative poles of embryos. We deal here with histochemical problems of an especially simple type.

But also some cases of more difficult histochemical problems may be treated in a fairly satisfactory way. In tissue the number of cells of a certain type may be taken as a sufficiently defined structural quantity if their cytological condition is uniform or varies from cell to cell (of this type) in a way which is satisfactorily defined by the general physiological state of the tissue. An example of this is the problem of enzyme distribution in a secretory organ like the stomach mucosa where it has been possible to relate quantitatively the distribution of pepsin to that of the number of chief cells in stomachs of moderately well-defined and constant physiological state. If the physiological conditions are altered certain structural changes in the cells following their life cycle or functional cycle have to be considered, and it is very difficult to find a quantitative measure of such changes. However, I may mention that in such cases reference quantities may be chosen such as the cell volume—if the cell volume changes in a well-defined way—or the portion of the cell volume not occupied by granules, or the number or volume (if measurable) of granules of a certain kind, and so on as far as the histological methods of differentiation will carry us.

Similar reference quantities have to be considered in the case of experiments on single cells. As an example the experiments by Holter may be mentioned in which a quantitative relation was found in single sea-urchin eggs between peptidase content on the one hand and cytoplasmatic volume not occupied by granules on the other.

Before entering into details of these problems attention must be directed to a certain technical difficulty in comparing histological and enzymatic investigations. Many structures of the cell are not directly observable in the fresh tissue but only after fixing and staining. Hence, in a number of cases the two kinds of observation cannot be made on the same piece of tissue because the process of fixation is entirely destructive to the enzymes and, reversely, because the tissue is made completely unsuitable for histological investigation by the enzymatic analysis. In the following a few examples will be given of how this difficulty may be overcome.

After this, I fear, somewhat sketchy attempt to describe the topic of my lecture today, I shall proceed to give some examples from the work carried out during the past nine years by Dr. H. Holter, the author and our co-workers in the Carlsberg Laboratory. I am sorry that time does not allow me to deal in detail with the methodical side of the question, but it may be sufficient to give a rough idea of the manipulations in order to make the basis of the following investigations clear. Three essentially different kinds of methods were employed, one of which is by far the most used, viz., the microtitration method developed initially by H. Holter and the author.[2,3,4,5,6] But in the last two years two other methods of investigation have been introduced, a dilatometric method developed in collaboration with Henry Lanz, Jr.[7,8] and a gasometric method developed in collaboration with David Glick.[9,10]

Fig. 1 shows in a schematic way the procedure in the titration method. By way of example a peptidase determination is described. A drop of water or dilute glycerine is introduced into a small reaction tube, volume 250 mm.^{3} The small piece of tissue is added by means of a fine glass needle. Five mm.^{3} of substrate (viz. an alanylglycine solution) are added. The micropipettes used are glass capillaries with two constrictions between which the volume is measured off. Next a stirrer is added consisting of a hollow and well-sealed glass bead filled with iron powder. The whole is mixed using an electromagnet with periodic circuit-breaker, the tube is closed with a stopper and immersed into a water bath at the temperature desired. When the enzymatic reaction has run for a suitable time it is stopped by adding alcoholic hydrochloric acid, then acetone with dissolved naphthyl red is added and the amino groups formed during the reaction are determined by titration with n/20 alcoholic hydrochloric acid, using a color standard for comparison. The stirring during titration is effected by means of the magnet with periodic circuit-breaker and the same small stirrer which remained in the reaction mixture all through the experiment. The burette is a capillary containing roughly 1-2 mm.^{3} per cm. and graduated in 0.2 mm.^{3} so as to allow an accuracy of reading of about 0.02 mm.^{3} Emptying and filling are effected by a fine screw at the lower end of the burette which forces a tightly fitting piston into an enlargement in the burette which is filled with mercury, thereby forcing a thread of mercury up into the capillary.

This method, modified for the specific purposes, has been employed

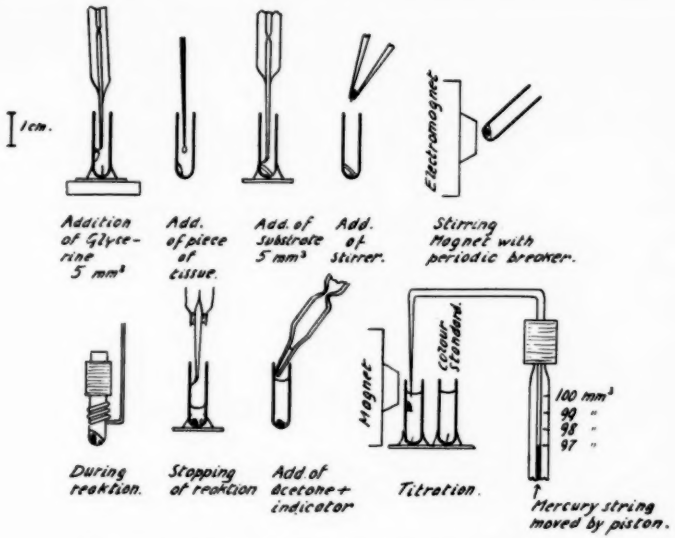

Fig. 1—Procedure in titrimetric estimation of enzymes.

for the determination of peptidases, proteinases, carbohydrases, esterases, etc. Its accuracy corresponds to $2.5 \cdot 10^{-9}$– $5 \cdot 10^{-9}$ mol of substrate split. The accuracy of enzyme estimation varies from enzyme to enzyme and is furthermore dependent upon the time of reaction. With long reaction times small quantities of enzymes may sometimes be estimated accurately, enzyme destruction being the limiting factor here. As an illustration it may be mentioned that $5 \cdot 10^{-9}$ mol of alanylglycine is split by one sea-urchin egg in one hour.

Fig. 2 shows the main procedure in the dilatometric method which is based upon the well-known fact that chemical reactions in liquid systems are followed by well-defined isothermal changes in density. The density change per unit of time may be used as a measure of the rate of reaction.

To the left in a small scale is shown a so-called gradient tube in which the density is measured. In the upper bulb there is a mixture of kerosene and bromobenzene with the density 0.99. In the lower bulb there is another mixture of the same liquids with the density 1.01 and in the connecting tube the density varies linearly from 0.99 to 1.01. The whole system is saturated with water at a suitable vapor pressure.

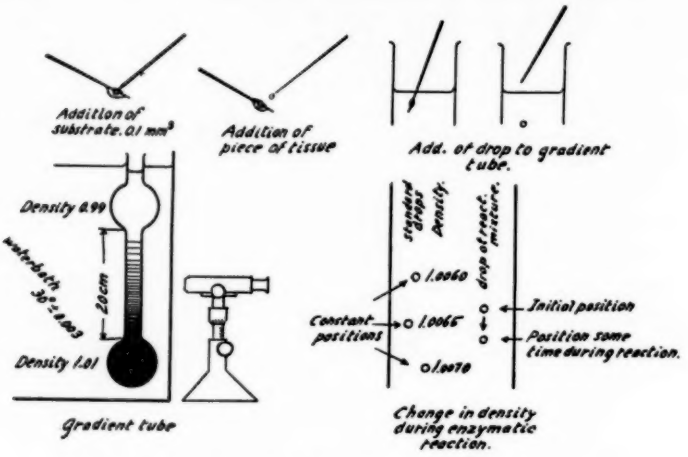

Fig. 2—Procedure in dilatometric estimation of enzymes.

If a drop of an aqueous solution is introduced at the top of the tube it will fall down through the medium and come to rest in a position where the density of the surrounding medium is identical with its own. Hence the density of the medium at any place may be determined by means of drops of known density and reversely the density of unknown solutions may be determined provided the gradient is standardized. The positions of the drops are measured in a horizontal microscope with micrometer.

The procedure in the enzymatic analysis is as follows. Again the peptidase determination may be used as an example. A drop of kerosene is put near the point of a glass needle. Into this drop a smaller one (0.1 mm.^{3}) of alanylglycine solution is introduced. The aqueous solution sticks to the glass and is entirely covered with kerosene. The piece of tissue is added with a fine glass needle or with a small pipette (e.g., in case of a single sea-urchin egg suspended in sea water). The needle with its two drops is introduced into the medium at the top of the gradient tube, the kerosene dissolves in the medium and the aqueous drop is removed from the needle by lifting it above the surface of the medium. The drop falls down through the medium and would reach a position of equilibrium after about fifteen minutes if the tissue added were free of peptidase. If this is not the case the alanylglycine within the

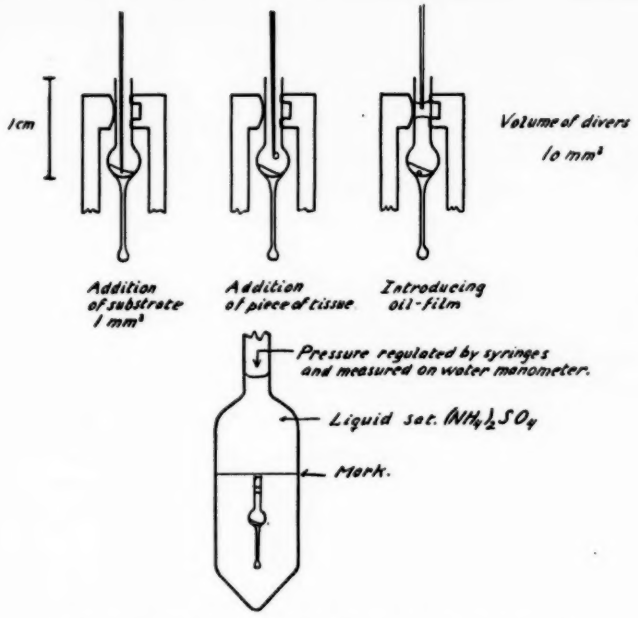

Fig. 3—Procedure in gasometric estimation of enzymes.

drop will be hydrolyzed and for each millimol of alanylglycine split, a volume of 9.4 mm.^{3} will disappear. The density of the drop will therefore increase, the drop will not keep a constant position but will continue to fall very slowly to levels with increasing density. The rate of the fall is a measure of the quantity of peptidase. This is shown in the figure. The drops to the left are 0.1 mm.^{3} aqueous standard drops of known density introduced simultaneously and serving to standardize the gradient in the neighborhood of the drop of reaction mixture.

The accuracy of the density measurement is about $3 \cdot 10^{-6}$. In a drop of 0.1 mm.^{3} this corresponds to the splitting of $0.3 \cdot 10^{-10}$ mol of alanylglycine. One sea-urchin egg will split this quantity of substrate in twenty seconds. That means that the peptidase content of 1/50 of a sea-urchin egg may be determined with an accuracy of a few per cent, provided all other sources of error are excluded.

Fig. 3 shows some details of the gasometric method. This method is based upon the principle of the cartesian diver well known from children's toys and from determinations of the density of liquids. In the

form employed for gasometric investigations the diver is a small thin-walled glass container, volume approx. 10 mm.^{3} Its gas space is closed by means of an oil film introduced into the neck. By adding or removing glass the weight of the diver may be adjusted so that at a pressure of 1 atmosphere it will just sink to the bottom of a vessel with saturated $(NH_4)_2SO_4$. This solution is chosen because it dissolves the ordinary gases only to a slight extent. At a slightly lower pressure, 20-40 cm. of water below one atmosphere, the buoyancy of the diver will counterbalance its weight and it is possible to adjust the pressure so as to bring the diver to swim at a given level (viz., the mark in Fig. 3) in the vessel and remain there for at least ten seconds. This equilibrium pressure is reproducible within 1-2 mm. water. If now for some reason the quantity of gas in the diver decreases or increases a corresponding change in equilibrium pressure will be observed and may be taken as a measure of the volume change since

$$\frac{\Delta v}{v} = \frac{\Delta p}{p}$$

where v is the gas volume of the diver and p the equilibrium pressure. An accuracy of pressure reading of 1 mm. water corresponds to an accuracy of volume determination equal to 0.001 mm.,^{3} since p and v are of the order of magnitude 10,000 mm. and 10 mm.^{3} respectively. A determination of carboxylase, for example, may be carried out as follows. By means of a fine micropipette 1 mm.^{3} of a sodium pyruvate solution is pipetted down into the bottom of the diver which is held in a vertical position by a suitable holder. Next, the piece of tissue is added and finally the film of paraffin oil. The diver is introduced into the vessel below and sinks to the bottom. After a few minutes a slight underpressure is put on the vessel and the equilibrium pressure is adjusted and read off. The readings are continued at suitable intervals, an empty diver serving as thermobarometer being measured at the same time. Between the readings the pressure on the diver vessel is brought back to 1 atmosphere. The change in equilibrium pressure corrected for the change in barometric height is then proportional to the quantity of CO_2 evolved. It is not necessary to stir during the reaction because the volume of the drop of reaction mixture is small as compared with its surface.

As an illustration it may be mentioned that the oxygen consumption of one sea-urchin embryo in 2 hours at 20° is just measurable with this method.

Experiments with such methods will—except in cases of more complex studies of metabolic processes (e.g., respiration)—furnish us with estimations of quantities of enzymes. The conditions at which the enzymatic reaction proceeds must be chosen so as to allow a calculation of the quantity of enzyme from the reaction rate. Here the histochemistry rests entirely on the results of general enzyme chemistry. It is important to point out that in most cases little or nothing can be said about the actual function in the cell of the enzyme estimated. An enzyme like peptidase estimated by measuring its catalytic effect on the hydrolysis of a peptide may in the cell have a quite different activity from that measured in the test tube at conditions most suitable for an *in vitro* experiment. It may even take part in synthetic processes.

Now concerning the sampling of tissue and cells, the requirement of histological control leaves two methods in the main:

1. *In vivo* observation of the histological structure of a given sample with succeeding enzymatic analysis of the same sample. If the object is not directly observable like sea-urchin eggs and other single cells of this type (protozoa, etc.), it is necessary to prepare the sample in a suitable form, e.g., in the case of tissue a thin slice in which counting of certain cellular elements or measurement of certain areas of homogeneous tissue may be carried out under the microscope.

2. Histological investigation on fixed and stained samples and enzymatic analysis of other non-fixed samples of the same object, under conditions assuring that the results obtained with the two sets of samples are mutually comparable. Figs. 4 and 5 show how this can be done in an exact way (Glick, Holter, Linderstrøm-Lang and Søeborg Ohlsen,[11] Linderstrøm-Lang and Mogensen[12]). A microtome of the Minot type is placed in a kryostat at −20°. It may be handled from the outside with two heavily lined leather gloves. A flat glass cuvette with warm water serves as a window and light is thrown down from the lamp above. A small cylinder with a diameter of 2 mm. is prepared from the tissue in question by means of a punch of a special kind. This cylinder is frozen on to the block of the microtome with its axis perpendicular to the plane of the block and cut in circular sections of suitable thickness (Fig. 5). In order to prevent the sections from curling up on the knife, which makes them unfit for histological investigation, a special device is constructed. Close against the side of the microtome knife farthest from the freezing block, a sheet of glass is placed and held at a distance of

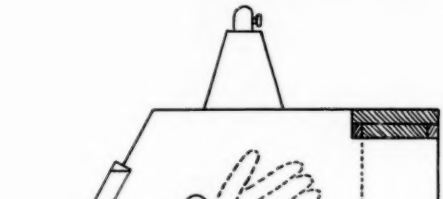

Figure 4

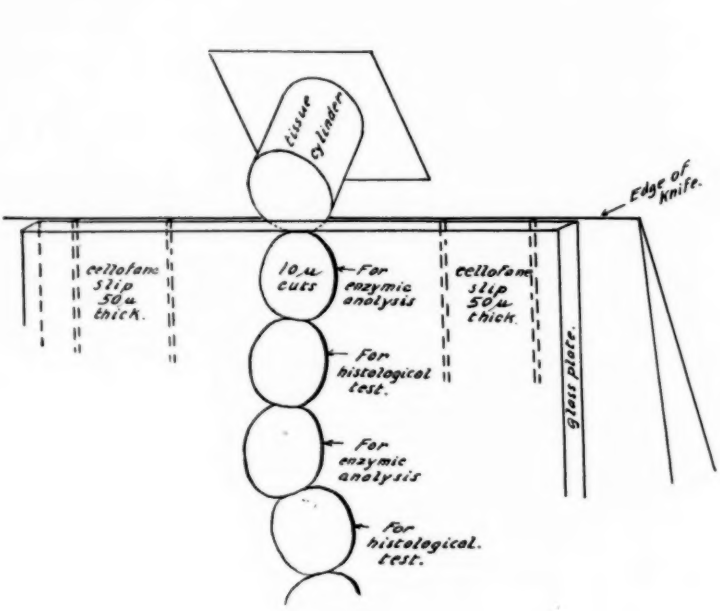

Fig. 5—Cutting of plane frozen sections of tissue.

50μ from the blade of the knife by means of two strips of cellophane. The sheet of glass can be swung back on an axis not shown in the picture and put in position again without displacement. It is important that the upper edge of the sheet of glass should coincide exactly with the edge of the knife. During the cutting process the glass is pressed down towards the knife and the sections slip between the sheet of glass and the knife and are thereby prevented from curling up. In this manner long series of plane 10μ frozen sections can be taken, the volume of which does not vary more than 5 per cent. Such sections are well suited for histological investigation after fixing and staining. The comparison with enzymatic analysis is easy, the general principle being that indicated in Fig. 5. Alternate slices are taken for the two kinds of investigation and the distribution curves of the enzyme on one hand and of any histological quantity on the other are graphically compared, the distances from the surface of the cylinder being plotted as abscissae.

Entering now upon the application of these methods I must apologize for the rather scattered character of what I am going to say. The construction of a tool which may be used for many different kinds of work necessarily leads to a testing out of the tool on many problems with little mutual relationship except the one that they may be attacked by means of the tool. My only hope is that in hearing about these applications you may discover that certain problems of your own might advantageously be taken up on this basis.

Histochemical Experiments

The problem of the function of the different cells building up the stomach mucosa is an old one and originates from the days of Heidenhain[13] and Langley.[14,15] The glands of the fundus part are built up in a way shown schematically in Fig. 6. At the top of the gland its wall is covered with epithelium cells. A little further down these cells give place to others, the neck chief cells and the parietal cells. Again deeper down the so-called chief cells appear while the neck chief cells disappear entirely and the parietal cells partly. Finally we pass beyond the basis of the glands and down into the muscularis mucosae, a fine membrane of connective tissue covering the outside of the stomach. Similar conditions are found in the pylorus part with the exception that the parietal cells are lacking. Due to this distribution of cells the mucosa has a definitely stratified structure and is exceptionally well suited for

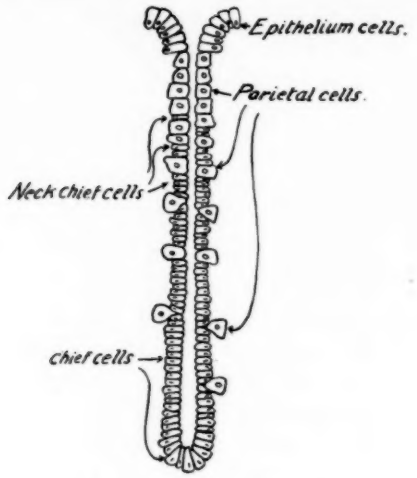

Fig. 6—Fundus gland of stomach mucosa.

investigations of the distribution of enzymes from the top to the base of the glands.

Fig. 7 shows the results of an investigation of this kind carried out six years ago in much the same way as just described (Glick, Holter, Linderstrøm-Lang and Søeborg Ohlsen[11]). I shall not enter into methodical details since recently many improvements of technique have been introduced. A small tissue cylinder is punched out of the frozen mucosa of a swine in a comparatively well defined state of digestive cycle. Circular sections of 25μ thickness are cut and sets of two sections form the samples for the pepsin estimation. The upper light curve shows the results of the enzymatic analysis, arbitrary pepsin units per unit of volume being plotted against distances of the samples from the surface of the mucosa.

The lower curve shows the distribution of the number of chief cells, parietal cells and epithelium cells + neck chief cells per unit of volume. The upper heavy curve is constructed with the assumption that 1,000 chief cells contain 2 pepsin units which correspond to $1.4 \cdot 10^{-3}$ mg. Parke, Davis pepsin (1:10,000). It is evident from the agreement between the two curves that pepsin is accumulated in the chief cells and with a rather constant mean quantity per cell. This is supported by

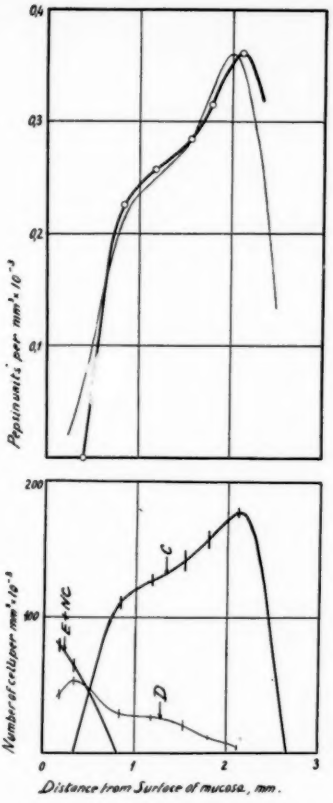

Fig. 7—Pepsin and cell distribution in fundus mucosa.

several other experiments of similar type but with other stomach samples.

It may again be emphasized that from these experiments nothing definite can be said about the state or activity of pepsin in the cells. The enzyme experiments were carried out under conditions where the full activation of pepsin was secured. From other investigations, however, we know that pepsin is present in its inactive form, as pepsinogen. Hence it is more correct to say that the mean quantity of pepsinogen per chief cell is constant and much larger than the quantity present in any other type of cell. We are therefore justified in assuming with Heidenhain and Langley that pepsinogen is manufactured and stored in the chief cells.

Pepsin is not lacking, however, in other types of cells in the stomach.

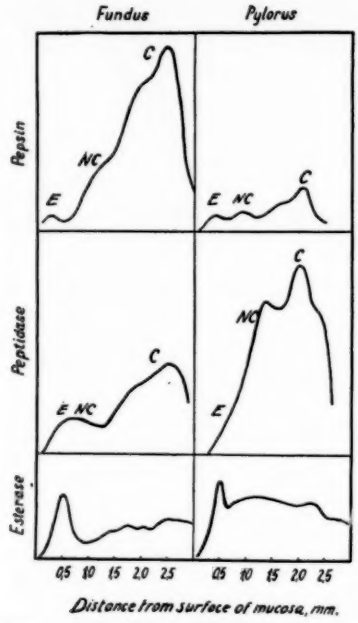

Fig. 8—Enzyme distribution in fundus and pylorus.

The epithelium cells and the neck chief cells contain some pepsin. Roughly 0.1–0.2 pepsin units are found per 1,000 chief cells in the pylorus and even in the duodenum very small pepsin quantities are found in the cells of the Brunner glands. This pepsin of other cells differs from that present in the chief cells of the fundus in being more firmly bound to the protoplasm of the cells. Its function is quite obscure. The connective tissue, muscularis mucosae, etc., are free of pepsin.

A special chapter is the distribution of dipeptidase. It is present in the glandular cells in all parts of the stomach. The chief cells generally contain most; a little less is found in the neck chief cells and least in the epithelium. Muscularis mucosae is peptidase free. Comparing the chief cells of fundus and pylorus it is generally found that the peptidase content is the higher, the lower the pepsin content. Fig. 8 shows this. The letters indicate the cells dominating in the given regions. Since dipeptidase has never been demonstrated in the secretions of the stomach it may be regarded as a typical endoenzyme, the function of which is intimately connected with processes taking place in the interior of the

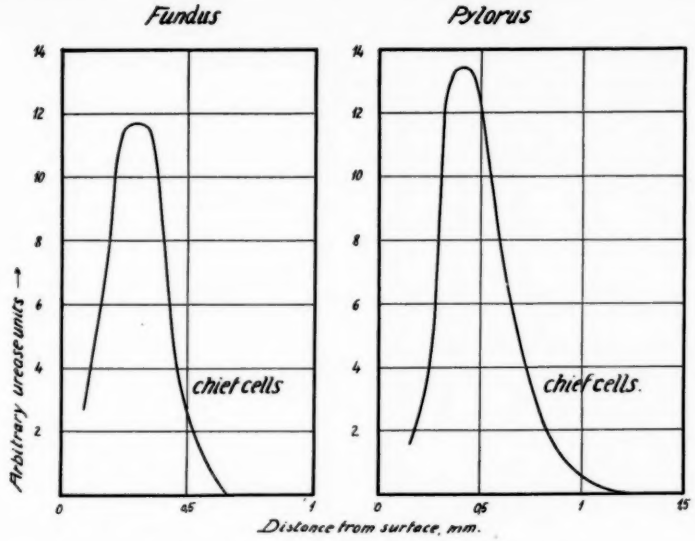

Fig. 9—Urease distribution in dogs mucosa.

cells of the secretory glands.

The curves at the basis of the figure show the esterase distribution (Glick[11]). A high peak in the epithelium region may especially be noted but the conditions are rather complicated due to the fact that the connective tissue including the muscularis mucosae is rather rich in esterase.

Urease could not be demonstrated in the mucosa of the swine. But it is present in great quantities in the mucosa of the dog as found by Luck[16]. The distribution is interesting in as much as it is entirely different from that of peptidase. From Fig. 9 it is seen that urease is accumulated in a region just below the surface epithelium and that the chief cells are free of this enzyme. It has not been possible to correlate its occurrence with that of any definite type of cell (Linderstrøm-Lang and Søeborg Ohlsen[17]).

As an example of the enzyme distribution in another animal organ an investigation by Weil and Owen Ely[18] may be mentioned. They studied the arginase distribution in the kidney of rabbits in much the same way as described above. Their results are seen from Figs. 10 and 11. The following types of cells were counted: The cells of the convoluted tubules; those of the ascending and descending Henle's loop tubules and of

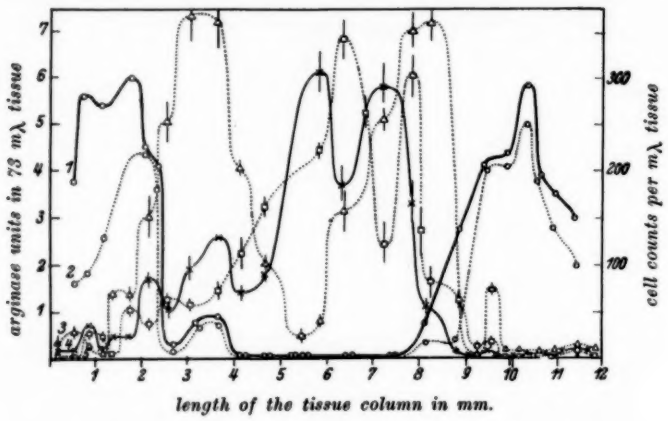

Fig. 10—Enzyme and cellular distribution in the medulla of rabbit kidney. Curve 1 shows the full activity of arginase; Curve 2, the initial activity of arginase; Curve 3, cellular counts of ascending Henle's loop tubules; Curve 4, cellular counts of descending Henle's loop tubules; Curve 5, cellular counts of collecting tubules. (Weil and Owen Ely[18]).

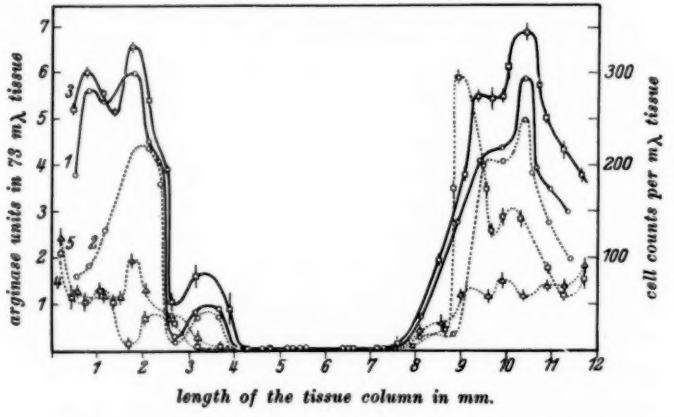

Fig. 11—Enzyme and cellular distribution in the cortex of rabbit kidney. Curve 1 shows the full activity of arginase; Curve 2, the initial activity of arginase; Curve 3, cellular counts of proximal convoluted tubules; Curve 4, cellular counts of distal convoluted tubules; Curve 5, per cent area of glomeruli tubules. (Weil and Owen Ely[18]).

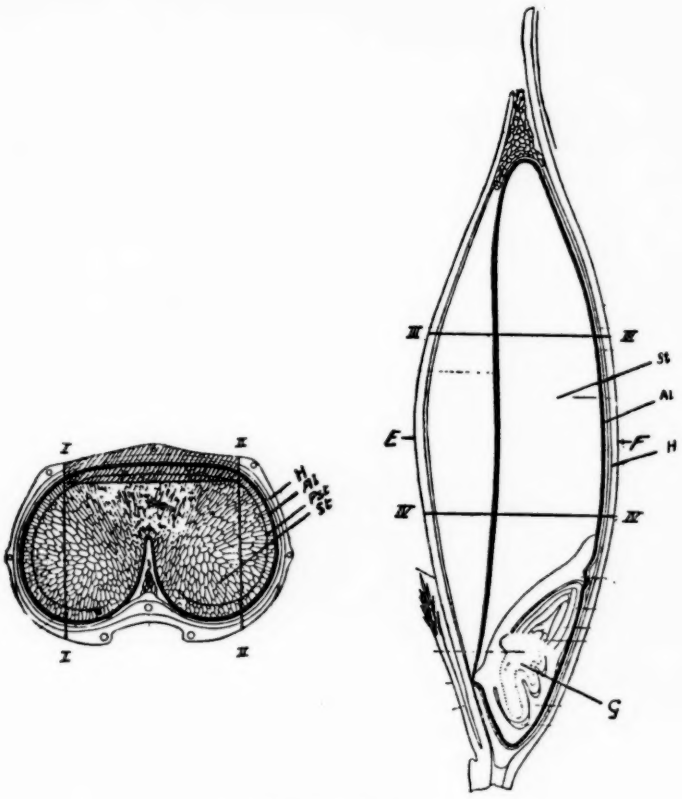

Fig. 12—Anatomy of barley grain.

Left: Transverse section E-F. I and II: limits of the cylinder. Al: aleuron cells. Pst: protein-rich starch cells. St: starch cells. H: husk. The hatched area was investigated.

Right: Longitudinal section. G: germ. III and IV: limits of the cylinder.

connecting tubules. The figures show that arginase is only present in the cortex and again here by far the best correlation is obtained between the distribution of arginase and that of the cells of the proximal convoluted tubules. This conclusive result, i.e., that the arginase is bound to these cells, is of great importance to the physiology of the function of the kidney.

Finally mention may be made of a short study of the amylase dis-

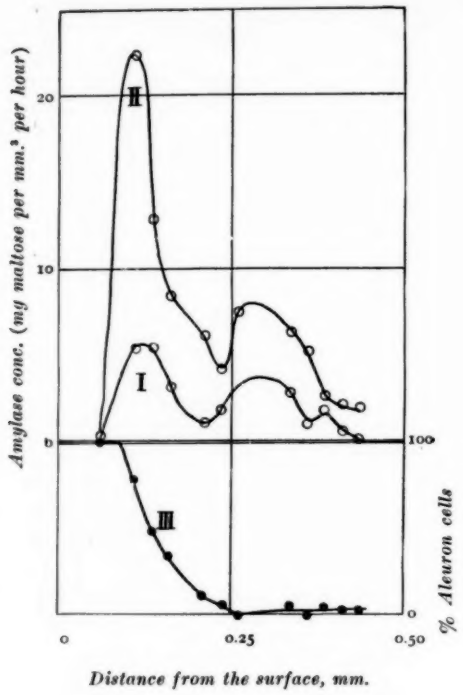

Fig. 13—Amylase distribution in barley grain.

tribution in the barley grain carried out in collaboration with C. Engel. Older experiments have shown that most of the amylase is found in the germ and here again accumulated in the palisade cells of the scutellum (Fig. 12). Another organ of the grain, the aleuron layer, is not so well known. It surrounds the endosperm except at the scutellum and borders inwardly on the starch cells. Its thickness varies from 1 to 3 cells. Some authors have found amylase in this layer, others have not.[19,20,21,22,23] The explanation is that the amylase is accumulated in a very thin layer of small protein-rich starch cells filled with embryonic starch granules. This layer is situated between the aleuron cells and the real starch cells of the endosperm both of which are poor in amylase. In previous experiments the aleuron cells were removed from the grain by grinding off the surface layers of say 50 grains. Hence it would sometimes happen that the amylase-rich cells were removed together with the aleuron cells

and sometimes not. Fig. 12 shows how the renewed investigation was carried out. Again a small cylinder was cut out perpendicular to the plane defined by the longest of the small axes and the long axis, and 25μ sections were cut, photographed between two small pieces of cover slides and afterwards investigated for β-amylase. This experiment may therefore serve as an illustration of a case in which the same sample is used for a rough histological test and for enzymatic analysis. It may be pointed out that the amylase activity per section is so great that extracts can be made of each section and aliquots used for the analysis. This enables us to determine both active and inactive β-amylase, the latter being activated by papain–HCN.[24] Fig. 13 shows a distribution curve. Curve I represents the distribution of active amylase, curve II that of active + inactive. At the base of the figure the distribution of aleuron cells is graphically given. The ordinates represent the area of aleuron in per cent of total area of the slice, both areas being measured planimetrically on the enlarged microphotographs of the sections. It is observed that amylase is missing in the aleuron cells and that the starch cells, the quantity of which increases towards the right, are also poor in this enzyme. The optimum is found just at the boundary between these two types of cells and a more exact histological investigation has demonstrated the presence of the above mentioned thin layer of small starch cells.

Cytochemical Experiments

The study of single cells such as are found in the tissue of higher animals is strongly impeded by their small size. Hence the cytochemist has to look for other objects sufficiently large to permit him to dissect them and investigate the pieces with the available micromethods. The eggs of certain marine invertebrates, e.g., sea-urchins have for a long time served as experimental material for the cytologists, and preliminary experiments[25] showed them to be extraordinarily well suited for quantitative microenzymatic investigations. Their size is of the order of magnitude 10^{-4} mm.^{3} A property of great importance is the conformity in behavior of eggs of the same batch. Upon fertilization they will cleave almost simultaneously when kept at optimal conditions. If centrifuged according to the method of Newton Harvey they will stratify and divide uniformly. Their enzyme contents are nearly identical. Hence results obtained with portions of one egg may be compared with

TABLE I

CONFORMITY OF PEPTIDASE CONTENT IN UNFERTILIZED EGGS OF THE SAME BATCH

SPECIES OF SEA-URCHIN	BATCH	NUMBER	QUANTITY OF PEPTIDASE IN ARBITRARY UNITS		MEAN
Dendraster excentricus	A	1	2.26	2.34	
			2.36	2.26	
			2.22	2.20	
			2.30	2.34	2.29
	B	1	1.68	1.76	
				1.60	1.68
Psammechinus miliaris	IV	3	4.31	4.25	
			4.25	4.11	4.24
	XII	3	2.46	2.50	
				2.70	2.55
Echinarachnius parma	A	1	3.36	3.16	
			3.04	3.26	
				3.30	3.22
	B	1	4.44	4.40	
			4.96	3.84	
			4.34	4.08	4.34
Paracentrotus lividus	A	1	5.94	5.82	
			6.30	6.12	
				5.92	6.02
	B	1	2.40	2.71	
			3.04	3.07	
			3.10	2.75	2.85

those obtained with another whole egg and the effect of the process of dissection may be controlled.

Table 1 shows the uniformity in peptidase content of eggs from the same batch. At the same time it appears that various batches may be quite different in this respect (Linderstrøm-Lang,[25] Philipson,[26] Holter[27] and unpublished data).

These eggs contain a number of formed bodies. There is the nucleus; a small body of chromatin. There is the yolk which fills up a considerable portion of the cell and may be regarded as "dead" material: protein, carbohydrate and fat serving as nutrition for the developing embryo. But in addition some granules are found which may be distinguished from the other formed bodies by their reaction with certain dyes. They have special interest to the cytologists and have several names. I shall

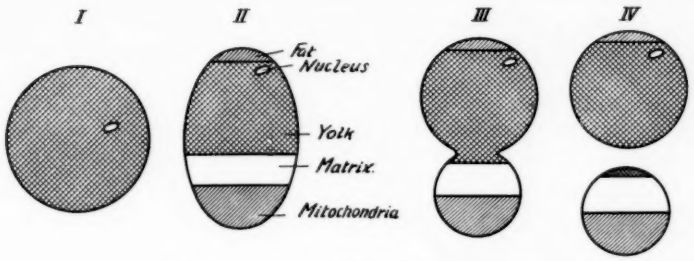

Fig. 14—Centrifuging of psammechinus egg.

refer to them as mitochondria. Their function in the cell has been much discussed and the plausible hypothesis has been advanced that they might be carriers of the enzymes of the cells (see Horning[28]). The following experiments carried out by Holter a couple of years ago[27] tend to show that this is not generally the case. In continuation of preliminary investigations by Philipson,[26] Holter studied the peptidase and catalase distribution in centrifuged eggs of sea-urchins. Fig. 14 shows the behavior of the eggs of the sea-urchin, *Psammechinus miliaris*, in the centrifugal field. The method used was that of Newton Harvey[29] according to which a density gradient is made in a small test tube by partial mixing of sea water with an isotonic sugar solution. Eggs introduced into this tube will collect in a layer having a density equal to their own. During centrifugation of the tube the eggs first become elongated and stratified. Later on they take the shape of a dumbbell and finally they divide in two spherical portions. The distribution of matter appears from the figure. It is evident that an investigation of the enzyme content of the two portions must give information about the role of the mitochondria as enzyme carriers. Holter's results were as follows: Neither in the case of peptidase nor in that of catalase was it possible to correlate the quantities of enzyme found with those of any of the formed bodies including the mitochondria of the cell. The only correlation obtained was that between quantity of enzyme and the cell volume not occupied by formed bodies, i.e., the volume of the cytoplasm proper, the matrix of the cell. The calculation of this volume is not easy and involves an estimation of the free space between closely packed particles, but using the figures collected by Harvey a satisfactory treatment was possible. Table II shows some of the results.

TABLE II

ENZYME DISTRIBUTION IN CENTRIFUGED EGGS

HOLTER (1936)

ANIMAL	OBJECT INVESTIGATED	TOTAL VOLUME RATIO L/H	MATRIX VOLUME RATIO L/H	ENZYME CONTENT IN ARBITRARY UNITS PER ONE EGG OR PORTION	PEPTIDASE RATIO L'/H'	L'H / H'L
	Peptidase					
Arbacia punctulata, Batch 1	Whole egg			0.446		
	Light portion, L Heavy portion, H	1.1	3.2	0.335 0.098	3.4	1.1
Arbacia punctulata, Batch 2	Whole egg			0.602		
	Light portion, L Heavy portion, H	0.6	1.2	0.323 0.249	1.3	1.1
Echinarachnius parma	Whole egg			3.22		
	Light portion, L Heavy portion, H	1.3	3.6	0.76 2.52	3.3	0.9
	Catalase					
Psammechinus miliaris	Whole egg			12.0		
	Light portion, L Heavy portion, H	2.9	0.9	5.5 5.3	1.0	1.1

Similar results have been obtained with amebae (Holter and Kopac[30]) and it seems to be a general rule that these two enzymes, dipeptidase and catalase, are found in the cells in a dissolved state. In the case of other enzymes like amylase, experiments by Holter and Doyle[31] have indicated an accumulation in formed bodies of the mitochondria type, but more experiments must be done before this question can be finally settled.

Before entering upon a short review of embryological work carried out with the methods described I should like to mention some investigations on the intestine of the nematode ascaris. The experiments are not concluded, but seem to open up possibilities for a study of the cytochemistry of secretion. The intestine of ascaris has a lumen of about 1.5 mm. Its wall is about 120μ thick and consists of one layer of long palisade cells oriented perpendicular to the plane of the wall. Fig. 15 shows a cut

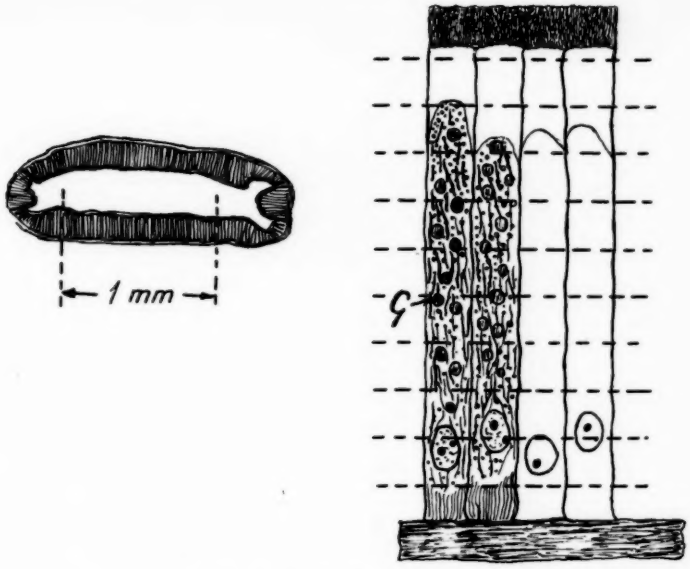

Fig. 15—Anatomy of ascaris intestine.

through the intestine and, to the right, a piece of the wall cut parallel to the long axis of the palisade cells. According to Hirsch,[32] it is possible in these cells to distinguish between different zones among which a central "working-zone" may be especially noted, characterized by the presence of so-called golgi bodies, G, and assumed to be the place where the digestive enzymes are synthesized. Due to the uniformity of the secretional cycle of the cells in question a fair agreement is found between their individual cytological pictures in a given intestine. Hence it is possible by cutting the wall parallel to its surface to obtain a series of sections the structure of which is a representation of that of the single cells (compare Fig. 15): To the left the cutting out of a circular disc of intestinal wall 1 mm. in diameter; to the right the positions of eleven 10μ cuts obtainable from the disc.

The cutting is very difficult due to the loose and mucous character of the tissue. Furthermore, the angle between the plane of the intestinal wall and that described by the edge of the knife during its movement relative to the tissue should not exceed 1:100, otherwise skew cuts with complex structure would be obtained. Time does not permit me to go

more fully into the special technique developed. I should like to mention that the disc of tissue is frozen between two 1 mm. thick gelatin films and cut in the kryostat described above. The films are cast on a plane mercury surface in order to secure parallel surfaces. So far our experiments have demonstrated nothing but the presence of dipeptidase at all levels of the cells. But we feel certain that a closer analysis including other enzymes will bring interesting results. In addition we think that the technique developed may be of value in experiments on other thin membranes in the organism.

Embryological Experiments

In the introduction mention was made of some experiments in which nature itself furnished the investigator with sufficiently defined samples for the histochemical analysis. In embryology the investigation is frequently restricted to a mere demonstration of chemical differences between the morphologically different parts of the embryo and does not include a comparison with more detailed differences in cytological properties.

As an example of such an embryological problem we may begin with the following simple question. Will the cells of a sea-urchin egg which has cleaved three times, with the formation of eight blastomeres, be identical with respect to their content of a given enzyme, say peptidase? We know that at this point of the development a differentiation has already taken place and that the two sets of four blastomeres are different from one another, their fate as animal and vegetative poles being determined. Preliminary investigations by means of the dilatometric method (Holter, Lanz and Linderstrøm-Lang[33]) have shown that the peptidase is distributed evenly among the blastomeres and evenly between the two halves (comprising four blastomeres each). Table III shows this. The variations are probably within the experimental error.

Holter and Lindahl (unpublished data) have continued the investigation with the very enzyme-rich eggs of *Paracentrotus lividus* using the microtitration methods. They found an equal distribution of peptidase in the embryo (Table IV) through all the stages of the development up to the pluteus stage. Since the peptidase content of the whole embryo remains constant during the same developmental period Holter and Lindahl draw the evident conclusion that the peptidase originally present

TABLE III

DISTRIBUTION OF DIPEPTIDASE IN EMBRYOS OF PSAMMECHINUS MILIARIS

Holter, Lanz and Linderstrøm-Lang[33]

BATCH	OBJECT INVESTIGATED	PEPTIDASE CONTENT IN ARBITRARY UNITS
1	Whole egg unfertilized	675
	One-half egg (4 blastomeres)	365
	One-half egg (4 blastomeres)	338
2	Whole egg unfertilized	950
	One-eighth egg	88
	One-eighth egg	96
	One-eighth egg	98
	One-eighth egg	117
	One-eighth egg	89
	One-eighth egg	95
	(Two-eighths being lost)	

TABLE IV

DIPEPTIDASE DISTRIBUTION IN EMBRYOS OF PARACENTROTUS LIVIDUS

Holter and Lindahl (1938)

STAGE OF DEVELOPMENT	OBJECT INVESTIGATED	CONTENT OF PEPTIDASE IN PER CENT OF THE CONTENT OF THE WHOLE EMBRYO	VOLUME IN PER CENT OF THE VOLUME OF THE WHOLE EMBRYO
16 cell stage	Animal half	50.6	50
	Vegetative half	49.8	50
Early gastrula	Animal half	51.5	50
	Vegetative half	46.3	50
Pluteus 60 hours	Ectoderm	81.6	82-81
	Entoderm	18.9	18-19

in the egg becomes equally distributed among all the cells of the embryo. Later on, of course, differences must occur since peptidase is not equally distributed in the fully grown animal. In this connection it is interesting to note that such differences have actually been found but with entirely different objects. Levy and Palmer[34] report the following unequal distribution in the case of chick-embryos:

Ectoderm arb. peptidase units per mm.^{3}..... 10
Mesenchym arb. peptidase units per mm.^{3}..... 3
Cerebral fluid arb. peptidase units per mm.^{3}..... 0

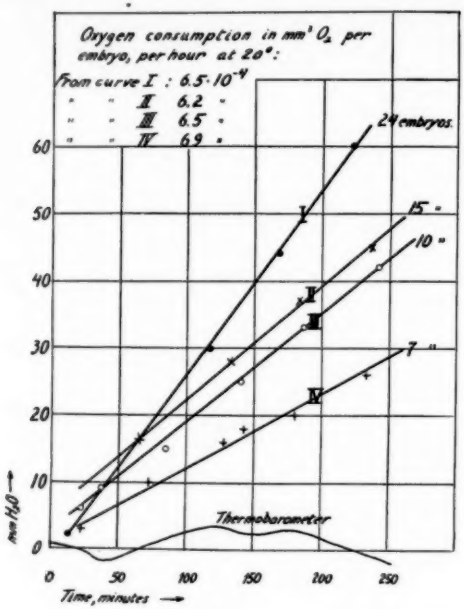

Fig. 16—Paracentrotus embryos (Holter and Lindahl).

Finally, an example of the application of the cartesian diver respirometer may be given. Studies on the respiration of embryos and parts of them have been carried out by Needham, Boell, Koch and Rogers;[35,36] by Brachet and Shapiro[37] and by Holter and Lindahl (unpublished data). Respiration may be studied with the diver technique of a film of dilute sodium hydroxide serving to absorb the CO_2 formed as introduced in the neck of the diver below the film of oil. The organisms are placed at the bottom together with 1 mm.³ of a suitable medium (sea water, etc.). The advantages of the method in the case of embryological investigations are its accuracy and the circumstance that no shaking is necessary because the embryos in swimming around will stir up the small drops sufficiently. In addition, as many as six divers may be tended at one time.

Fig. 16 shows one of Holter and Lindahl's experiments with different numbers of whole embryos of *Paracentrotus lividus* in the early gastrula stage. The ordinates represent the pressure changes observed on the manometer corrected for changes in the atmospheric pressure. Each

TABLE V

RESPIRATION OF ANIMAL AND VEGETATIVE HALVES OF EMBRYOS OF PARACENTROTUS LIVIDUS

Holter and Lindahl (1938)

HOURS AFTER FERTILIZATION	NUMBER OF EMBRYOS OR PORTIONS OF EMBRYOS	OBJECTS INVESTIGATED T = WHOLE EMBRYO A = ANIMAL HALF V = VEGETATIVE HALF	OXYGEN CONSUMPTION IN $MM^3 \times 10^{-4}$ PER EMBRYO OR PORTION OF EMBRYO PER HOUR AT 20°
5	16	t	3.6
	15	a	2.0
	16	v	2.0
11	19	t	4.9
	30	a	2.8
	27	v	2.6
11	33	a	2.6
	36	v	2.2
	37	a + Lithium	1.3
	35	v + Lithium	1.3

millimeter corresponds to a volume change of about one thousandth of a $mm.^3$ varying a little with the individual gas volumes of the divers. The time is represented in the abscissa. It appears that the respiration proceeds approximately linearly with time during the period of observation. Furthermore, closer calculation shows that the oxygen uptake per embryo per hour is the same independently of the number of embryos in the diver.

Experiments of this type carried out with animal and vegetative halves of embryos failed to demonstrate any difference in rate of respiration between these two portions of the embryos. Furthermore, the percentage inhibition by lithium ions was found to be identical in the two cases (Table V). It is evident that these results, though negative, will be of considerable value in the discussion of the relation between metabolism and differentiation.

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SOME COMMENTS ON ARTERIOSCLEROSIS IN WILD MAMMALS AND BIRDS*

HERBERT FOX

THIS opportunity is appreciated not only because it uses in your symposium some of the work that has interested us at the Penrose Laboratory of the Philadelphia Zoological Society and the Division of Comparative Pathology of the University of Pennsylvania, but also that it expresses your desire to know something of spontaneous pathology in other than human animals. This phase of study is still neglected, though less so than when Metchnikoff, forty-five years ago, wrote that pathology is almost the only subject of study in which the comparative method has been ignored. We could add that, even today with wisely selected methods, comparative pathology might many times lead to safer conclusions than would some of the bizarre mental and physical gymnastics of the experimentalist.

The first comment that can be made on our subject is that arteriosclerosis occurs in practically all vertebrates below man. We are concerned now with birds and mammals. Many Reptilia and a few Amphibia have been seen with this condition of the vessels. The second comment is that not only has arteriosclerosis been seen in menagerie specimens but also in material killed in the wild, that is, during the animal's free life.

As introductory to the form of disease seen in birds and mammals, it is desirable to define the forms that are recognized, which must be concise in such a short presentation, even though it appear dogmatic.

The simplest of arteriosclerotic change is intimal hyperplasia which amounts to a great excess of the normal widening of the intra-elastic layer that occurs in all animals as age advances. The second step in vascular disease seems to be atheroma, an excess of lipoid matter in the thickened intima, often minimal, at other times as in the monkey and eagle, dominating the statistical record of the variety. The next step is

* Read May 16, 1939, at The New York Academy of Medicine before the Section of Medicine.

degeneration in the media, be it simple hyalinization or as hardening when calcium is laid down. The greatest development is of course, atherosclerosis, a combination of all the essentials of the foregoing divisions.

The next comment that can be made is that all forms of arteriosclerosis occur in the lower animals. The most prominent lesions are in the major vessels but the small branches and of course arterioles, are sometimes similarly affected. The differing animal groups display their peculiarities of type, however. It is perhaps proper at this point to emphasize that I am speaking only of wild animals, not domestic ones that represent but few zoological groups and moreover have been artificially reared by man, prostituted to his use and are more truly wild in Nature's scheme of things than are ferocious beasts.

It is proper at this place to see the different lesions as they are found in wild animals according to the system already described.

In preparation for this presentation I have studied the available material since my last and general article that covered seven thousand animals. The results are almost identical as to type and distribution. The pictures amply reveal the damage in the arterial wall that can be accepted as nearly identical with the disease as known for man. Some phases of the process, such as renal or coronary sclerosis, do not lend themselves well to lantern slide reproduction and are at no time advanced; of this more later.

Table I demonstrates how the material was studied and may be used here to call attention to a few points for the appropriate comments. The figures for the Carnivora show that they have a tendency to intimal thickening and medial disease and that they give the highest figures for involvement of the small arteries, including vascular nephritis. This is not the case for the split-toed ungulates which have an arterial degeneration of the same type.

Attention to the birds easily discovers that the anserine and psittacine birds lead the list; others may have a higher percentage but have not supplied enough examples to deserve a sharp emphasis.

Real analysis comes from the totals for mammals versus birds. Here we see that arteriosclerosis has occurred at about the same percentage in birds and mammals, 15.4 and 13.7. The average exhibition time for the whole group respectively, sixty-one and sixty-nine months, and for those that had arteriosclerosis, respectively 134 and 137 months, is about as one is to two; the vascular group is certainly well ahead of the general aver-

TABLE I

ARTERIOSCLEROSIS IN MAMMALS AND BIRDS IN THIS STUDY

Animal Orders or Families	No. for Study	No. Arteriosclerotic	Percentage	Average Exhibition Time for Affected Orders	Average Exhibition Time for Arteriosclerotics	No. Intimal Hyperplasia	No. Atheroma	No. Medial Degeneration	No. Atherosclerosis	No. Coronary Disease Arteriosclerotic Type	No. Myocardial Disease	No. General Nephritis	No. Vascular Nephritis	No. Small Artery Disease
Cercopithecidae (Old World Monkeys) ..	30	6	20	74	181	2	2	1	2	2	4	2		2
Cebidae (New World Monkeys) ..	10	2	20	35	81		2				1	1		
Carnivora	71	9	12.6	81	183	4		3	1	1	1	2	4	4
Rodentia	47	5	10.6	29	79	2		3	1		1	3		1
Artiodactyla (Split-toed)	69	13	18.8	87	146	6	2	9	1		3	6	1	1
Total Mammals .	227	35	15.4	61	134	14	6	16	5	3	10	14	5	8
Passeriformes (Nesting or singing birds of passage)	234	16	6.8	60	106	1	5		9	1	4	1		2
Piciformes (Woodpeckers) ...	7	1	14.2	61	77		1							
Coraciiformes (Toucans)	8	3	37.5	87	222		1		2		1			1
Psittaciformes (Parrots)	114	25	21.9	75	91	6	15	1	6		7	2		1
Strigiformes (Owls)	12	2	16.6	31	77		2							
Acciptriformes (Eagles, etc.)	15	3	20	49	147		1	1	1		1			
Anseriformes (Ducks, etc.)	66	21	31.4	96	128	1	8		12	3	5	3	1	3
Ardeiformes (Herons, storks, etc.)	13	3	23	100	116	1	3							
Balearicifformes (Cranes)	10	1	10	101	408				1					
Galliformes (Ground fowl)	100	5	5	33	134		3	1	1		1			
Total Birds	579	80	13.7	69	137	9	39	3	32	4	19	6	1	7

TABLE II

ANIMAL ORDERS OR FAMILIES IN WHICH NO ARTERIOSCLEROSIS HAD BEEN SEEN AT THE PENROSE LABORATORY

Primates	
Pongidae (gorillas and other apes)	20
Hylobatidae (gibbons)	13
Hapalidae (marmosets and lower Simians)	188
Chiroptera (bats)	12
Lipotyphla (hedgehogs, etc.)	10
Coccygiformes (cuckoos)	41
Phoenicopteriformes (flamingoes)	23
Charadriiformes (plover group)	40
Lariformes (gulls)	55
Sphenisciformes (penguins)	32
Ralliformes (gallinules and coots)	105

age. The average for those with arteriosclerosis being very good for menagerie maintenance, one might ask if the disease is good for the animal or is it just our usual reasoning that the animal has chanced to live long enough to get it. Is arteriosclerosis such a baneful form of the irreversibility of tissue?

The pathological features of mammals and birds are revealed in the totals, the immediately outstanding of which are as follows: mammals exceed sharply in intimal thickening and medial disease while birds have greater atheroma and full-fledged atherosclerosis. It must be recalled, however, that mammals do have this final form, as exemplified by the seal, the prominent exceptional case of which has been shown.

We have been dealing with so-called arteriosclerotic-positive varieties in menageries and it is fair to see the negative ones, Table II. Perhaps, had more examples been available, cases would have been found. We have seen the distribution of the disease in the animal scale, from primates to ratite birds and equally we see absence. We see quiet and restless varieties, we see carnivores and herbivores in both, we find that all spots of the zoogeographical earth are represented. It is notable that the bat and the gull are free and the passerine has much. If there be a group that is more affected than another, the quietly moving or the restless, it would probably be the former; the gentle duck and the dignified swan offer good examples; the busy-body monkey is not greatly affected.

TABLE III

LIST OF 'TRULY WILD ANIMALS' FROM THE WILD THAT PRESENTED EVIDENCES OF ARTERIOPATHY

Chimpanzee	Intimal hyperplasia and atheromatoid swelling of the aorta
Chimpanzee	Atheroma of arch
Moustached Monkey	Slight medial degeneration
Phalanger	Medial musculo-elastic degeneration and free fat, in aorta and large extremity vessel
Tanager	Early medial disease
Thrush	Early medial disease
Parrakeet	Medial degeneration at root of aorta in sinus of Valsalva
Buzzard	Atheroma of lower half of aorta, with fibrillation of elastica
Buzzard	Medial degeneration of aorta near heart; atheromatoid thickening of intima at arch and upper thoracic

Mammals studied	63	216
Birds studied	153	

One of the early comments was to the effect that arteriosclerosis occurs in "truly wild" as well as in menagerie animals. Table III shows a list of lesions found in animals sent to me by hunters and museum students in their naturalistic field work. The list is short and the percentage of findings is low but the essentials of arteriosclerosis can be discovered in animals uninfluenced by the so-called refinements of civilization. The lesions are not different from those discovered by a study of menagerie specimens.

Having surveyed the material upon which comments can be made, it is suitable that these remarks be continued by those that relate to our ideas of causation and effect. To approach a causation of a change one must know what the normal is and what it is at the various stages of youth, maturity and senility. Blood vessel walls, in common with other tissues pass through a change in structure. There occur a thickening of the intima, a fibrillary multiplication of elastic strands, a disarrangement of the elastic, muscular and cellular elements of the media, with possibly the appearance of fat. At least we believe that these are the accompaniments of inversive or irreversible anatomy and therefore of chemistry. These occur in man and lower animals. And there is nothing in the

gross and microscopic characters of arteriosclerosis that may not find its *qualitative* counterpart in the aging artery. The conditions then are differences of degree, or it may be more appropriate to state that those conditions that we call senile irreversibility of arteries merge into the disease of arteriosclerosis. This general condition holds for the lower animals but at varying rates or degrees. The conditions that affect the rate and severity of the process, such as medial degeneration of the cerebral human arteries or the major vessels of the bovine, are certainly acceptable as varied. The intimal overgrowth in the feline, the atherosis of the human coronary or the full-fledged process of the anserine vessel may be pushed along by another set of influences. Can we learn by comparative anatomy, physiology and pathology anything that would help to understand these inversive processes? Unfortunately little, but let us see. There are so many factors that their nature and influence must be sketched only.

The mammalian artery is closely similar throughout the Class albeit the carnivorous animals seem normally to develop heavy intimal layers at a younger age, appear to have a most generous elastic supply and a more robust muscular tissue than is the case with herbivores. In carnivores intimal hyperplasia stands first, then followed by muscular damage. The herbivores proper, bovines and cervines, for example, have as their major mural change, medial degeneration and then intimal hyperplasia; this might be related also to their more slender elastic supply and relatively thinner vascular wall. The intima of mammals appears to have greater power of growth and seems to be more often pathologically damaged.

Standing out in the relationship of anatomy and arteriosclerosis is the condition in birds. At the location where the most pronounced lesions are found in the bird, the aorta, a true well-formed internal elastic lamina does not exist. The intima is very delicate and normally grows little. The wall as a whole has relatively more elastic tissue and less muscle. The smaller vessels, as they become paler yellow-blue, have much muscle and an irregular strand of elastica just beneath the very delicate intima.

The bird with this vascular construction has atheroma and fully developed atherosclerosis in the major vessels and very little change in the smaller vessels. A very common seat for atherosclerosis in the bird is just above the heart, a place that feels the impact of the oncoming blood stream perhaps more than does any place of any vascular system

of any other animal. This force is given by a heart that is two and a half times the weight of that of a mammal of the same body weight.

These anatomies and these lesions occur. Are there variables in the anatomy of the human being that, in the presence of other factors favoring arterial degeneracy, predispose to the different forms of human disease? I would not mean that he who has renal arterial intimitis is a carnivore, he who has intermittent claudication from Mönkeberg's sclerosis is a herbivore or he who has full atherosclerosis is a bird, but their vessels might be abnormally constructed so as to favor these lesions.

Conditions of body, of size, of living characters, of sex, of known pathological panels such as susceptibilities to infectious disease and tumors, frequency of malnutrition, nostalgia, tractability, all these have no measurable effect upon the incidence of arteriosclerosis. That ever debated subject, diet, with its constantly shuffled alphabetical new deal, may have; let us hope that some one will find a capital V for vascular disease.

The food of the specimens of the first study was very high in carbohydrates, poor in protein, low in fat and ash and very irregular in vitamin contents. The second series received a balanced ration built around a formula of protein, two; fat, one; carbohydrate, five; ash, five; fortified by extra vitamins, by fruits, grains, vegetables and growing seeds. Despite the better balance of ration, greater vitamin content resulting in better exhibition specimens, arteriosclerosis has not changed essentially in appearance or distribution.

Comparative pathology is only good when it is applicable, as in our present case, to human medicine. The lessons from the vascular pathology of mammals and birds are limited but they exist. Perhaps it is fair to begin by emphasizing the principal differences between human and wild animal disease. Man has a small percentage of acutely fatal pathological processes but because he can nurse himself back to recovery, his major change may be called chronic pathology. Wild animals, especially as we are forced to know them best, die quickly when ill and their pathology is chiefly acute. Chronic cardiac, pulmonary, renal and similar morbid processes are uncommon. I think focal infection occurs in all animals, high and low, but I am loth to diagnose it.

For these reasons and some minor ones, the effects of vascular disease upon morbidity and mortality of wild animals are difficult to estimate. Insofar as atherosclerosis of the major vessel is concerned, it seems to

stand in exactly the position it occupies in human medicine. In man it assists in the end of senility. In lower animals it does the same. On a few occasions we have seen in Paradise birds atheroma and atherosclerosis in the sinus of Valsalva and rupture of the heart nearby. The worst cases of complete atherosclerosis, however, did not die from the vascular condition. They were among the examples that lived in the longest year-life brackets.

The Gull and Sutton concept is the one that requires our consideration and for this purpose we shall review the facts of the kidneys, heart and brain where the grave human lesions develop. What has been found has been at autopsy, our medical acumen and investigative facilities not reaching a nephrosclerosis in a bear.

Renal sclerosis of vascular type was found in 26 of 229 cases of the first series and in 6 of 115 of the second. I am not over-confident of the accuracy of the first figure but at all events the incidence of this form of arteriolar change is low. This sclerosis of the small vessels was associated with atherosclerosis of the large ones only in 6 of the total 32 nephroscleroses.

Coronary disease was diagnosed in the two series, nine times in all, of which three occurred in Paradise birds, and was almost certainly the cause of death. With none has there been a true infarction or major scar. Discovery of the extent of the lesion in small birds is difficult and has rarely been entirely satisfactory. Thrombosis on a roughened coronary place has been seen under the microscope but not grossly. Coronary shock can scarcely be called a frequent event in wild animal pathology.

Comment upon myocardial disease *per se* is best confined to our last series. Twenty-eight of the 115 arteriosclerotic bodies showed this lesion, usually as muscular fibrillation and fine fibrosis but also, notably in the old baboons and in parrots, a scar perceptible to the eye. True heart failure has not occurred from it.

Cerebral arterial disease can be dismissed with a few words. Never have we seen a massive cerebral hemorrhage of the apoplectic type. Many brains have been studied for diseased vessels without success. But then this is true with human material especially if one would try to find a vessel that is bad enough to break. On several occasions small arteries with vacuoles under the elastica or distorted as if the whole wall were about to become autolysed, were seen but no damage about them was found. Certainly none that suffered intimal herniation was seen.

Here we have a survey but not all the picture of arteriosclerosis in wild birds and mammals, an irreversibility of tissue chiefly in the major blood vessels, much less and with few dire consequences in their small vessels. The incidence is low, very much lower than for human beings. If the process be comparable to man's and become prominent as age advances, is it consistent to think of them both as inversive changes, that of man assisted by other chronic pathology? It is not to be forgotten that man suffers much chronic pathology, notably from infection and metabolic alteration, conditions quite rare in lower animals. And finally in this contrast, man and possibly lower animals suffer constant changes of blood pressure, a subject too extensive for this review, that can participate in the whole group of causes of tissue degeneracy.

A final comment would seem to be that arteriosclerosis is a failing of all animals and, as man has the most of it, perhaps he is the poorest.

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PSYCHOLOGICAL STUDY OF THE MIGRAINOUS SYNDROME*

HERMAN SELINSKY

THIS psychological study of the migrainous syndrome is based upon material gathered from interviews with 200 patients who suffered for a number of years from recurrent paroxysmal headache, invariably hemicranial. These headaches were diagnosed as migrainous in character; for in addition to the characteristic headache, there were one or more accompanying symptoms: nausea, vomiting, visual disturbances, chilliness, and urgency in urination and defecation. None of these patients showed any signs of organic disease of the central nervous system. There was a ratio of four females to one male in this series. The presence of such factors as familial predisposition and allergic tendencies existed in the patients' histories to a high degree and were regarded as evidence of constitutional background. It is taken for granted that a physico-chemical mechanism serves as the organic vehicle for this syndrome. The investigations of Wolff and Graham[1] have demonstrated that the headache is related to a cranial arterial dilatation which usually can be controlled by ergotamine tartrate.

This communication has a two-fold purpose. First, to draw attention to the fact that the migrainous syndrome can be materially benefited by psychotherapy. Despite the writings of Touraine and Draper,[2] Harold Wolff,[3] Olga Knopf[4] and others[5] who stress psychological factors, only two or three of these 200 patients whom I examined had possibly ever been approached previously from this therapeutic viewpoint. The other purpose of this paper is to attempt a dynamic concept of this psychophysiological problem. In passing, attention is called to a statement which Jelliffe[6] made about the migrainous syndrome that my own experience has confirmed, namely, that the migrainous attack occurs in the general population much more commonly than is usually recognized. Dr. Wolff tells me this is also his impression.

* Read April 11, 1939 at The New York Academy of Medicine before the Section of Neurology and Psychiatry.

To illustrate the thesis of this paper, I shall cite briefly the history of a patient, as the pattern delineated in this simplified form is rather typical of that which is so often found in migrainous subjects.

The man, who is forty-one years of age, is employed as a decorator only seasonally. He is unmarried and lives with his widowed mother, his father having died when the patient was a child. The patient has suffered from periodic hemicrania for many years, but this has become extremely frequent and distressing for the past six months. The basic sources of his emotional disturbance are: 1. A constant fear of insecurity (connoting anxiety). 2. A morbid sense of responsibility about supporting his mother. He resents this burden and yet has not been able to modify his attitude about it. It is significant that he quarrels with his mother when he is not employed. His demeanor during the interview is that of an inhibited person who is rigid and guarded.

Let us study the train of events during a typically severe attack. The paroxysm is ushered in by a slight dulling of the intellect; then there is a dull pain over one part of the head, usually frontal, not uncommonly just above one eye. There is a feeling of dread about the advancing attack. The headache gradually increases in intensity tending to spread over a large area (hemicranial). The sufferer finds increasing difficulty in continuing his work as the headache proceeds; his intellectual faculties are more restricted as he becomes torpid and irritable. He finds light and sound disagreeable to an extraordinary degree so that he seeks seclusion in a quiet and dark room. He may express an offensive reaction to an odor in the room. He is chilled; his hands and feet become cold; and he may have tingling or numbness of the fingertips. Commonly he experiences nausea and often vomiting. He may have increased sphincter irritability, that is, frequency with urgency of urination or defecation or both; or he may have constipation which he may attempt to relieve by a cathartic or an enema to rid himself of some offending substance which he holds responsible for his ailment. Washing out the sinuses sometimes has a similar motive, i.e., to remove an offending substance. He may experience visual disturbances such as scintillating scotoma, but more often he complains of a blurring of vision. He may have a sensation of giddiness, and sometimes a ringing in the ears. If possible, he resorts to sleep, which usually alleviates the attack.

The entire picture is one in which the individual is graphically expressing an extraordinary inability to master some disturbing situation.

The attack indicates a markedly hyperesthetic reaction of the human organism to environmental pressure and to pressure from within. It seems that the peripheral sensory apparatus which is the mechanism that enables the organism to perceive, explore and thereby adapt itself to the environment is being strained to the point of agony.

It is reasonable to assume that great difficulty in meeting a situation may arouse mixed feelings of anger, discouragement, frustration and fear. Such reactions, in fact, are found on intensive study. Since he cannot master the environmental situation, the patient must find some compromise. This compromise may be considered to be achieved by the attack which makes it justifiable and necessary to withdraw from the confronting problem which he finds painful or intolerable.

Thus each symptom expresses dramatically the fearful helplessness and frantic effort to reject something offensive and insufferable to the ego of the subject which constantly is seeking harmony: to find a satisfactory balance between desires of the self and the environmental restraints.

To this theoretical explanation of the attack, the objection may be raised that this contention really stretches credibility. How can one know that the headache serves such a purpose? To this question, it must be admitted that one cannot be certain, but, if we view the behavior of human beings as systems of psychobiological adaptations, then the migrainous attack may be considered a protective device. This device is not desirable nor adequate, but it is the only adaptation which the individual can effect at that particular time with his equipment, which may be circumscribed by inhibitions as well as by biological limitations. The migrainous paroxysm is a faulty protective device, to be sure, but what shall we say of such a protective device as sham death which is observed in lower forms of life! One is reminded of the reaction of unicellular organisms such as the Paramecium or amoeba: the organism attempts to master the object in its path by engulfing or incorporating it; but should it prove undesirable, ejecting it or withdrawing entirely from it. As a psychobiological phenomenon, the migrainous attack represents an attempt to eject, reject and withdraw; the loss of consciousness marks the most intense effort to withdraw.

It is far from rare to get a history of at least one fainting spell following a migrainous attack when a survey is made of the individual's experience; and with some patients, loss of consciousness is a frequent mani-

festation. Among the papers of Hughlings Jackson[7] and Gowers[8] is found an opinion that migrainous and epileptiform states are related phenomena. H. A. Riley[9] has observed this relationship in his extensive clinical studies of migraine.

In the explanation proposed by Kardiner[10] to account for the syncopal reaction of the traumatic neurosis (complete disability of the executive ego functions induced by the repressed fright so that the adult individual feels helpless in attempts at mastery of the situation), one may find guidance in studying the migrainous reaction. This view is particularly reinforced by the clinical pattern in the patient who is experiencing increasing disability because the headache and its accompanying symptoms have become a daily burden. It is at this stage of the disorder that fainting spells are apt to occur. The daily headache indicates that the intolerable situation has remained unmastered and that the struggle is more intense (rebellion at the offending situation, yet inability to overcome it).

I have seen several instances of reaction to a difficult situation in which fainting attacks give place to constant headache as the external or internal pressure is somewhat relieved. For example, a woman had suffered from intense guilt feelings when her child, whom she had tried to do away with by taking various abortifacients, died soon after birth. Subsequently, as she was able to formulate an alternative explanation for the child's death, namely, incompetent obstetrical care, the fainting spells ceased, but she then suffered from constant headache. Also thereafter she remained sexually frigid.

In the migrainous subject we are dealing with an inhibitory reaction to an aggressive impulse which permits the suffering subject to withdraw from this situation. This psychophysiological reaction strongly suggests what Rado has so aptly termed the "riddance urge." The suffering of the patient also is conducive to evoking sympathy from others rather than angry retaliation which might be provoked by an assertive action.

Again, the role of the special senses is stressed for purposes of recapitulating the psychophysiological concept. These special senses serve defensive and aggressive aims in the organism's adaptation to the environment. They form part of the integrative apparatus of the nervous system. But, unlike more primitive species, the human being in our society cannot freely utilize motorial aggression against the challenging environment because there are moral or social forces which inhibit such impulses. Therefore anxiety is provoked with the fear of being over-

whelmed by the environment: overwhelmed if he remains quiescent, or, if he attempts violent action to overcome the offending source. The need for repressing such impulses repeatedly brings about a state of conditioned inhibited response and this provokes a migrainous attack, i.e., a retreat. The dulling of intellectual faculties which heralds the paroxysm may be regarded as a cortical inhibition due to the anxiety aroused, whereas the self-directed raging irritability of the organism can be hypothecated as a subcortical excitatory reaction. In these instances in which anger precedes an attack, the headache may be regarded as having a defensive or self-punishing aim. This seems to be a sort of apologetic expression in having lost one's self-control. Self-control is invariably a source of great pride in the migrainous subject; it is significantly related to the rigidity of the personality structure.

These paroxysmal headaches are particularly frequent in women with intellectual leanings and inhibited behavior. A very common situation is the role of migrainous headache in the life of the harassed housewife. The moral or ethical forces which have been inculcated in the upbringing of such women have made it impossible for them to deliver themselves of burdens which they inwardly rebel against, e.g., the bearing and raising of children under adverse economic conditions, and the constant drudgery of house work.

My observations coincide with the personality studies of migrainous patients by H. G. Wolff,[3] Touraine and Draper,[2] Knopf,[4] and others who have pointed out that the migrainous subject is often of an intellectual type with tendency to worry, rigidity of attitude, driving ambition, exaggerated sense of responsibility, marked sensitiveness to criticism, and meticulous attitude towards work or responsibility. Furthermore in women, there is usually impairment of ability to achieve sexual gratification. The thinking and behavior of those migrainous patients whom I have studied reveals what one might call an obsessional tendency. What is the significance of these characteristics? Each signifies a defensive trait and is a defense against a constant anxiety which is belied by the apparent self-assurance and self-control which migrainous subjects display. In fact, the attempt at self-control has operated to develop a rigidity about standards of achievement and behavior which originally were intended for defense against the basic insecurity but now have made the subject a prisoner within himself.

Touraine and Draper believe that the bout of headache represents a

struggle in the migrainous individual who is trying to "escape from the mother's influence yet there was a compulsion not to leave her." This view is somewhat in accord with F. Fromm-Reichmann's[11] hypothesis; she says, "Migraine is a physical expression of unconscious hostility against consciously beloved persons."

The insecurity which is the nuclear framework in the emotional background of these patients often produces dreams in which they express a longing for parental protection. But is it not a psychological symbolism which refers to the patient's desire to find more adequate support in his search for security from pressing problems which confront him? There is a struggle for independence with the spectre of insecurity intimidating the patient on the one hand and the individual urge to achieve freedom on the other. So that one may see alternating episodes of submissiveness or aggressiveness recurring in the mood of the subject as expressions of exaggerated reactions to these conflicting impulses.

These individuals display an excessive sense of responsibility which makes its appearance early in life, so that, for example, they are inclined to act as the main support for younger siblings or for ageing parents; or to contribute voluntarily to the support of indigent relatives. From this, they receive an ego gratification which incidentally it is difficult to make them see and accept as part of thir motivation. Their basic need has been to strive inordinately and uncompromisingly for security, for it was regularly discovered in this study of migrainous subjects that they had experienced intense feelings of insecurity in their childhood. The insecurity may have been derived from a fatherless or motherless home, so that the child was given responsibility prematurely; or may have been engendered by sibling rivalry. In their strenuous attempts to adapt themselves to environmental handicap, they have reacted with character formations which gave them such appellations as "serious," "hard-working," "self-sacrificing," "good-hearted," "lacking in ability to play or relax." The women patients almost uniformly resented being female and naturally suffered a great deal during the menses. Many of them were frigid in their sexual reactions. The idea of self-control appeared dominant in their reactions to problems and this habit of repression led to a structural rigidity of emotional forces which prevented them from "letting themselves go." There was a frequent expression of hostility in women patients when referring to the mother, as though the patient felt that the mother's influence had been harmful or inhibiting. And, in fact, this resentment

could be justified when the mother's attitude was seen to be harsh or excessively repressive or apprehensive about the daughter's behavior.

Summary

The migrainous reaction represents a psychophysiological reaction to a state of tension induced by a mental struggle or conflict. In this struggle, anxiety and resentment are prominent emotional forces, stirred up by the effort of the subject to master a presenting situation. There is a basic sense of insecurity in the background which the individual attempts to control by conscientiousness, ambitious striving and exaggerated sense of responsibility. The attack seems to represent inability on the part of the patient to react with mastery or decisiveness to some challenging problem. The seizure may be considered a physical expression of inhibited (unconscious) protest made in a morbid manner. The migrainous reaction is an ailment which often can be benefited by psychiatric study and therapy. The degree of psychotherapeutic success depends upon the patient's intelligent and persevering coöperation. It is our duty to apply accumulated psychiatric knowledge to the relief of these patients.

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DEATHS OF FELLOWS

GIBB, WILLIAM TRAVIS: 400 West End Avenue, New York City; born in Bellefonte, Pennsylvania, December 29, 1862; died in New York City, July 6, 1939; graduated in medicine from New York University Medical College in 1886; elected a Fellow of the Academy April 4, 1901.

Dr. Gibb was an instructor in chemistry at New York University Medical College, 1882-87, and in gynecology at that institution, 1887-95. He was gynecologist to the St. Elizabeth's Hospital, consulting gynecologist to the City Hospital and consulting surgeon to the Central and Neurological Institute, Welfare Island.

Dr. Gibb was a Fellow of the American College of Surgeons, a Fellow of the American Medical Association, a member of the Society of Medical Jurisprudence and a member of the County and State Medical Societies. He was the author of many works on gynecology and surgery.

HEITZMANN, LOUIS: 38 West 90 Street, New York City; born in Vienna, Austria, May 14, 1864; died in New York City, July 9, 1939; graduated in medicine from the College of Physicians and Surgeons, Columbia University, in 1885; elected a Fellow of the Academy June 5, 1890.

Dr. Heitzmann was emeritus professor of pathology of the New York Medical College and Flower Hospital, consulting physician to the Lenox Hill Hospital, a Fellow of the American Medical Association, a member of the American Urological Association and a member of the State and County Medical Societies.

STRATTON, EDWARD AUGUSTUS: 112 Liberty Street, Danbury, Connecticut; born in Danbury, Connecticut, November 8, 1861; died in Danbury, Connecticut, July 9, 1939; graduated in medicine from New York University Medical College, New York, in 1883; elected a Fellow of the Academy January 3, 1907.

Dr. Stratton was consulting surgeon to the Danbury Hospital, a Fellow of the American College of Surgeons, a Fellow of the American Medical Association and a member of the State and County Medical Societies.

CORRECTION • APRIL, 1939 BULLETIN

DIAGNOSTIC SIGNIFICANCE OF CHANGES IN LEUKOCYTES

M. M. WINTROBE

Table VI, on page 235 of the April, 1939, Bulletin is corrected to read as follows:

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TABLE VI

CAUSES OF LYMPHOCYTOSIS

1. Certain acute infections: pertussis
   infectious mononucleosis
2. Exanthems, after the initial stage, especially in mumps and German measles
3. Chronic infections; such as tuberculosis, secondary and congenital syphilis, undulant fever
4. During the stage of convalescence from an acute infection
5. Exophthalmic goitre (usually only relative lymphocytosis)
6. Infants and young children, especially in the presence of rickets and malnutrition
7. Lymphatic leukemia